

# Spinal beta3-Containing GABA(A) Receptors Mediate Pre- and Postsynaptic Effects of Etomidate

Christian Grasshoff, M.D.,<sup>1</sup> Rachel Jurd, Ph.D.,<sup>2</sup> Uwe Rudolph, M.D.,<sup>3</sup> Bernd Antkowiak, Ph.D.<sup>1</sup> (Abstract No.: A-1115)

<sup>1</sup>Section of Experimental Anesthesiology, Eberhard-Karls-University, Tuebingen, Germany.

<sup>2</sup>Ernest Gallo Clinic and Research Center, University of California, San Francisco, California, USA.

<sup>3</sup>Laboratory of Genetic Neuropharmacology, McLean Hospital, Harvard Medical School, Belmont, Massachusetts, USA.



## Abstract

**Background:** The ablation of spontaneous and stimulus-induced movements by general anesthetics is largely mediated in the spinal cord. In case of etomidate beta3-containing GABA(A) receptors account predominantly for these effects.[1] We have shown recently that the efficacy of intravenous anesthetics in depressing spinal neurons is limited.[2,3] This limitation results from a presynaptic mechanism of action, reducing the release of GABA.[3] In the present study we used cultured spinal slices obtained from these mutant mice to test whether GABA(A) receptors containing a beta3 subunit account for pre- or postsynaptic effects of etomidate.

**Methods:** Organotypic spinal cord tissue slices were prepared from pregnant beta3 (N265M) mice (day 13-15) according to the method described by Braschler and used for experiments after 12 days in vitro. The effects of etomidate on spontaneous action potential firing were investigated by extracellular voltage recordings from ventral horn interneurons. Furthermore, spontaneous inhibitory post synaptic currents (sIPSCs) of GABA(A) receptors were conducted by the means of whole cell recording. All procedures were approved by the animal care committee and were in accordance with the German law on animal experimentation.

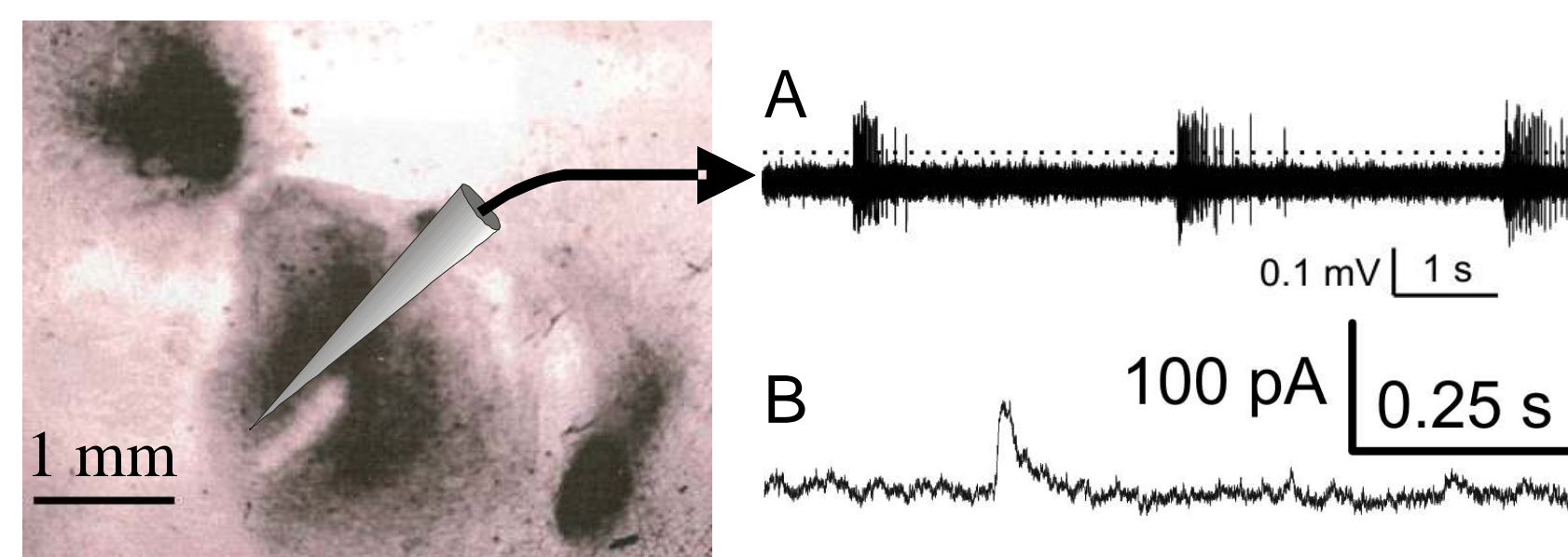
**Results:** Extracellular recordings demonstrated that the depressant effects of etomidate on spinal neurons were abolished in organotypic slices from beta3 (N265M) mice in the concentration range from 0.25 to 5  $\mu$ M. Furthermore we used whole cell recordings to investigate the effects of etomidate on sIPSCs caused by GABA(A) receptors. Decay times of sIPSCs as a measure of postsynaptic effects were prolonged in the wild type concentration-dependently (1.5  $\mu$ M, 92.3 $\pm$ 5.8 ms; 2.5  $\mu$ M, 118.2 $\pm$ 6.5 ms) but only slightly affected in the mutant (1.5  $\mu$ M, 30.3 $\pm$ 2.5 ms,  $p$ <0.001; 2.5  $\mu$ M, 43.1 $\pm$ 2.2 ms,  $p$ <0.001). The frequency of sIPSCs as a measure of presynaptic effects of etomidate was depressed concentration-dependently in the wild type (1.5  $\mu$ M, 43.5 $\pm$ 4.7 %; 2.5  $\mu$ M, 57.0 $\pm$ 4.6 %) but not in slices from mutant mice (1.5  $\mu$ M, -0.7 $\pm$ 8.4 %,  $p$ <0.001; 2.5  $\mu$ M, -1.7 $\pm$ 6.0 %,  $p$ <0.001).

**Conclusions:** Our results demonstrate that depressant effects of etomidate on spontaneous network activity were completely abolished in slices obtained from beta3 (N265M) mice. These data are in good accordance with the effects of the anesthetic in vivo.[1] Since the mutation affected both, the frequency of sIPSCs and IPSC current decays we conclude that pre- and postsynaptic GABA(A) receptors incorporate beta3 subunits in spinal ventral horn interneurons.

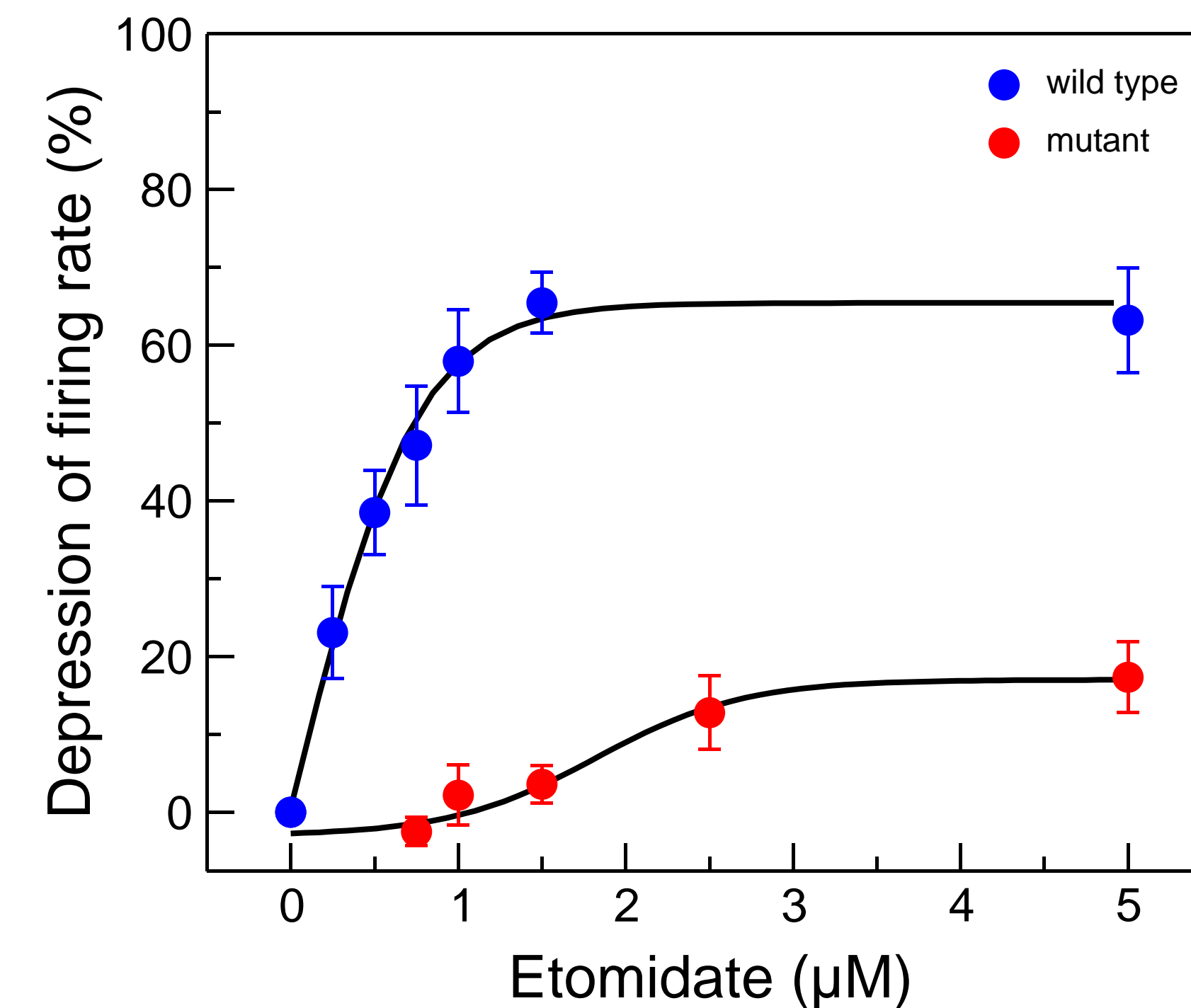
### References:

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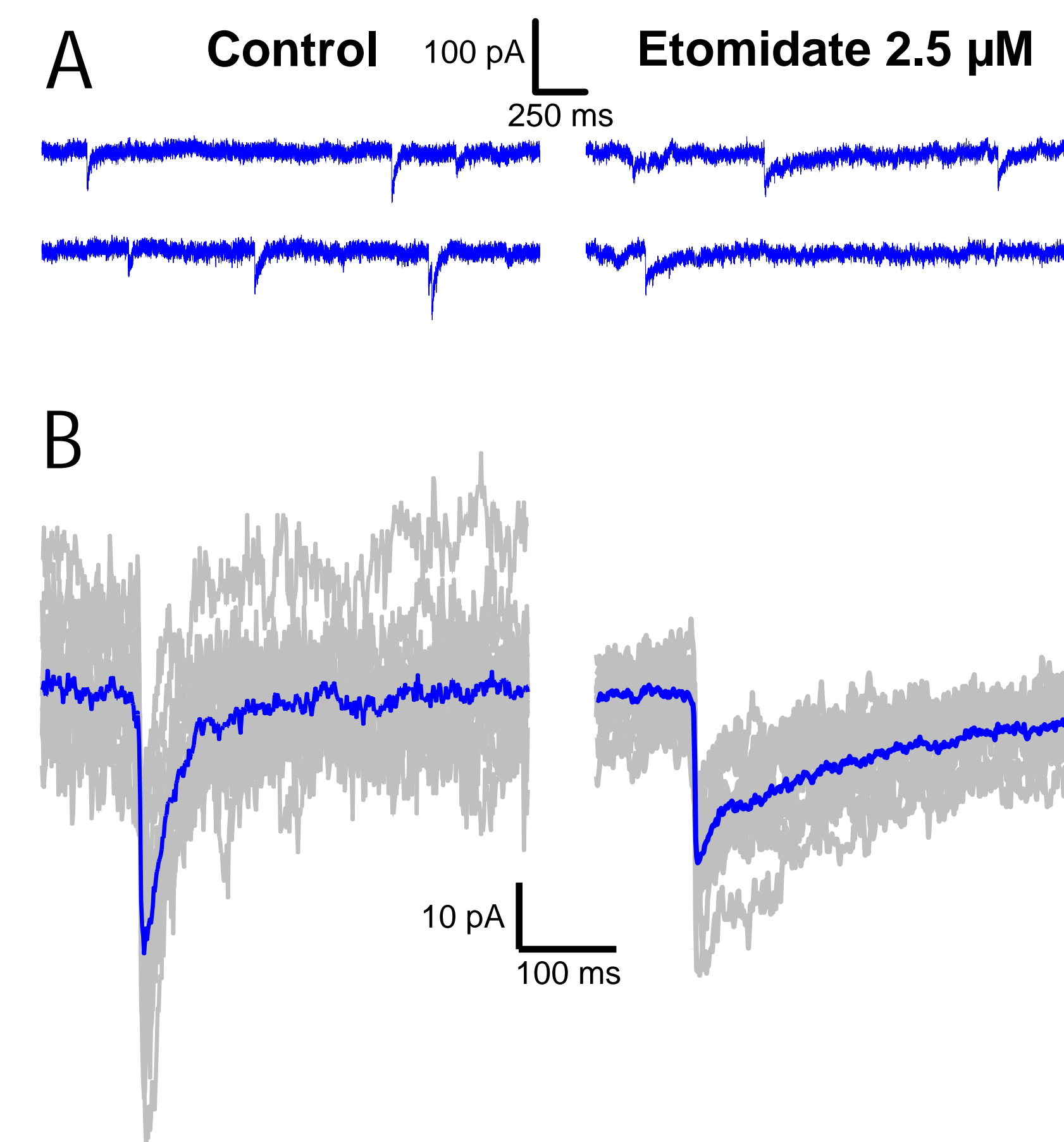
## Methods



**Fig. 1:** Recordings were performed on ventral horn interneurons in spinal cord-dorsal root ganglia cocultures after two weeks in vitro. (A) Extracellular recordings. Action potentials appeared in bursts, separated by silent periods. The broken line indicates the threshold for event detection. (B) Miniature inhibitory post synaptic currents (mIPSCs) of GABA(A) receptors obtained from a ventral horn interneuron.

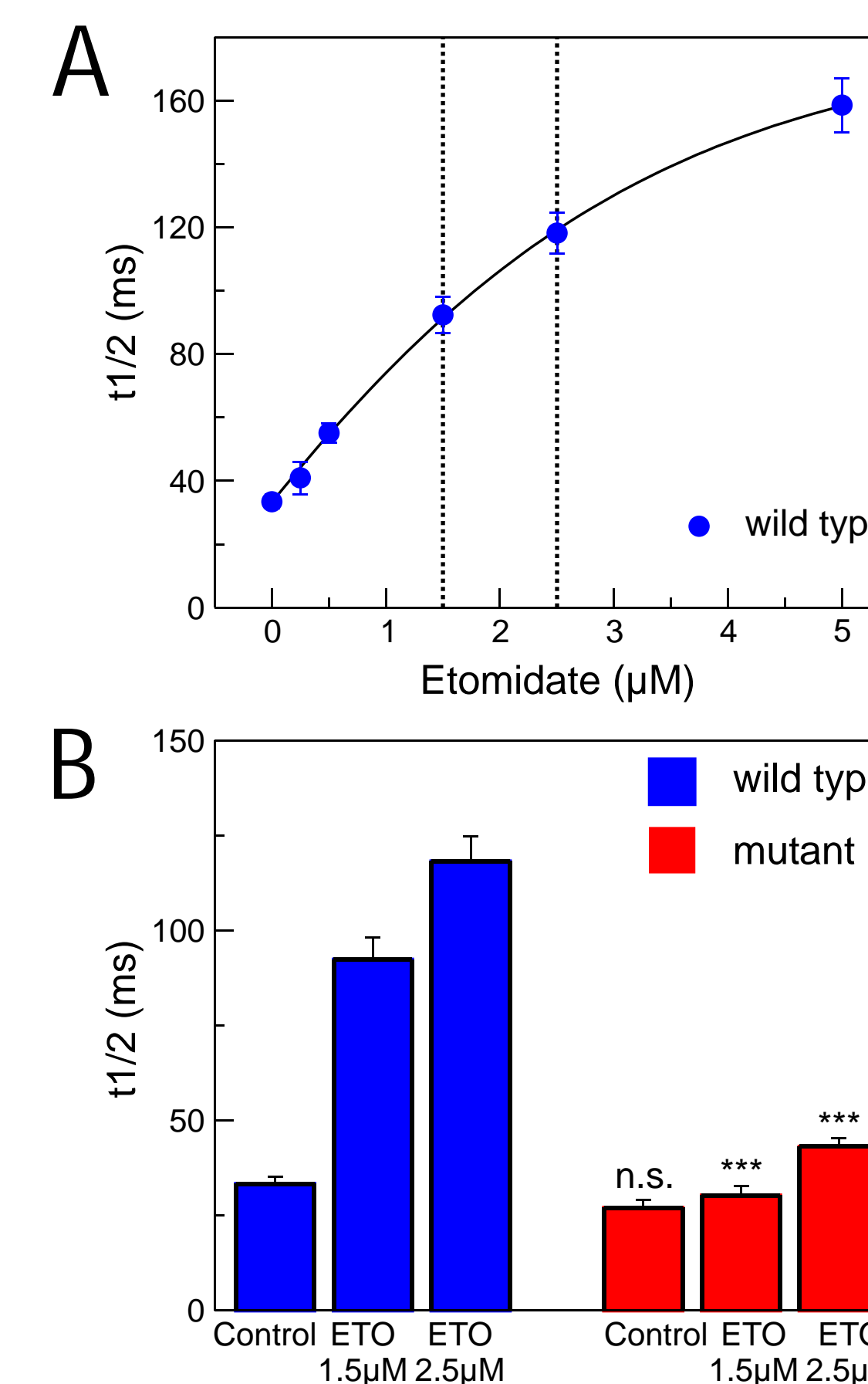


**Fig. 2:** Concentration-response relations of etomidate-induced depression of mean firing rates in **wild type** and **beta3 (N265M)** mice. For each concentration, the mean value and standard error were obtained from 6-12 cells. The effects of etomidate were calculated by comparing the spike rates before and during treatment. The curves were fitted by Hill equations.

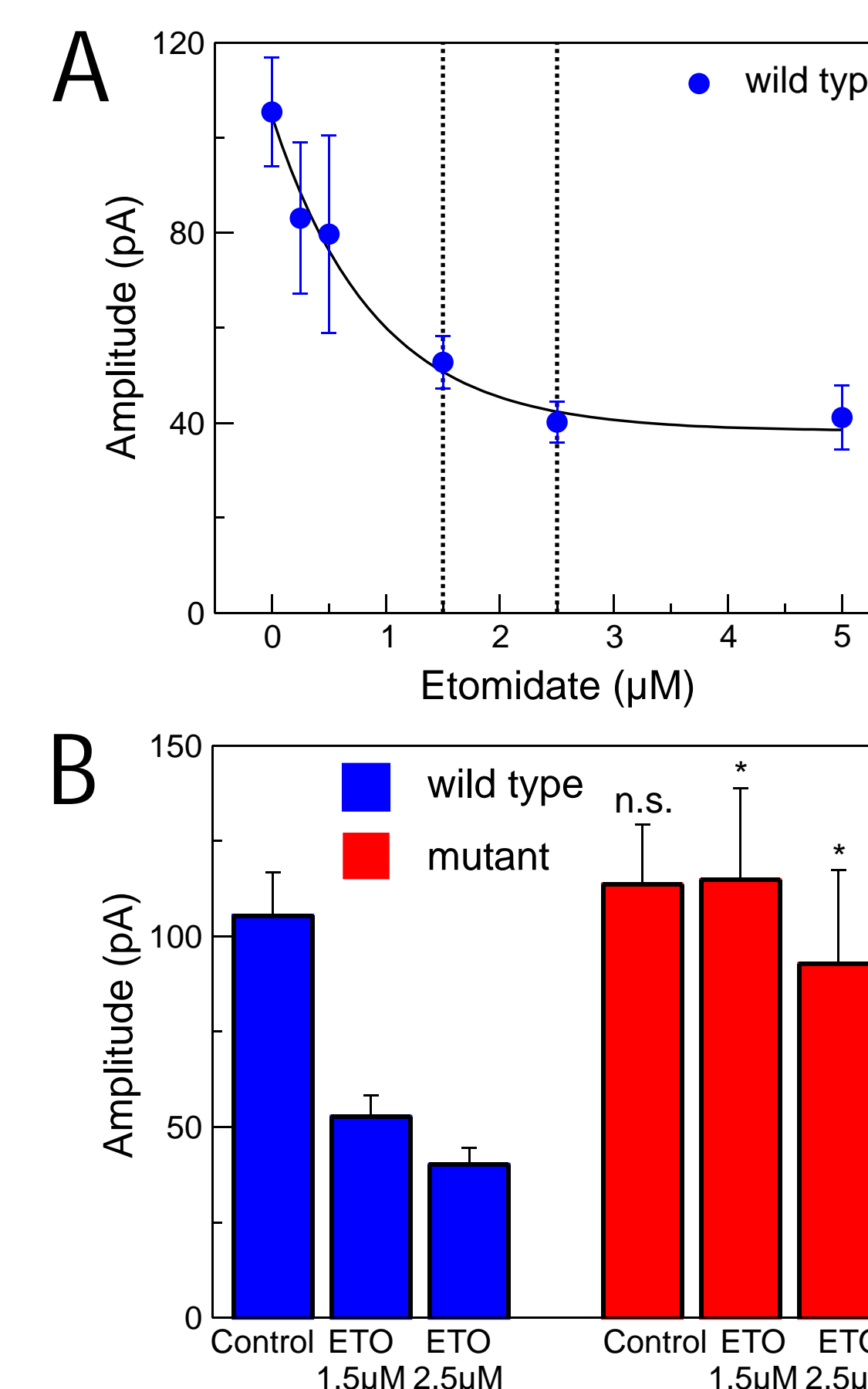


**Fig. 3:** Original recordings of spontaneous inhibitory post synaptic currents (sIPSCs) measured from wild type animals in the absence and presence of 2.5  $\mu$ M etomidate. CNQX 50  $\mu$ M, AP5 50  $\mu$ M, and strychnine 1  $\mu$ M were added to the bath medium. The cell was held at a membrane potential of -70 mV. Patch pipettes were filled with the recording solution (in mM) 145 CsCl, 1 MgCl<sub>2</sub>, 5 EGTA, 10 HEPES, and 2 MgATP. (A) Original recordings. (B) Overlay of spontaneous IPSCs shown in (A).

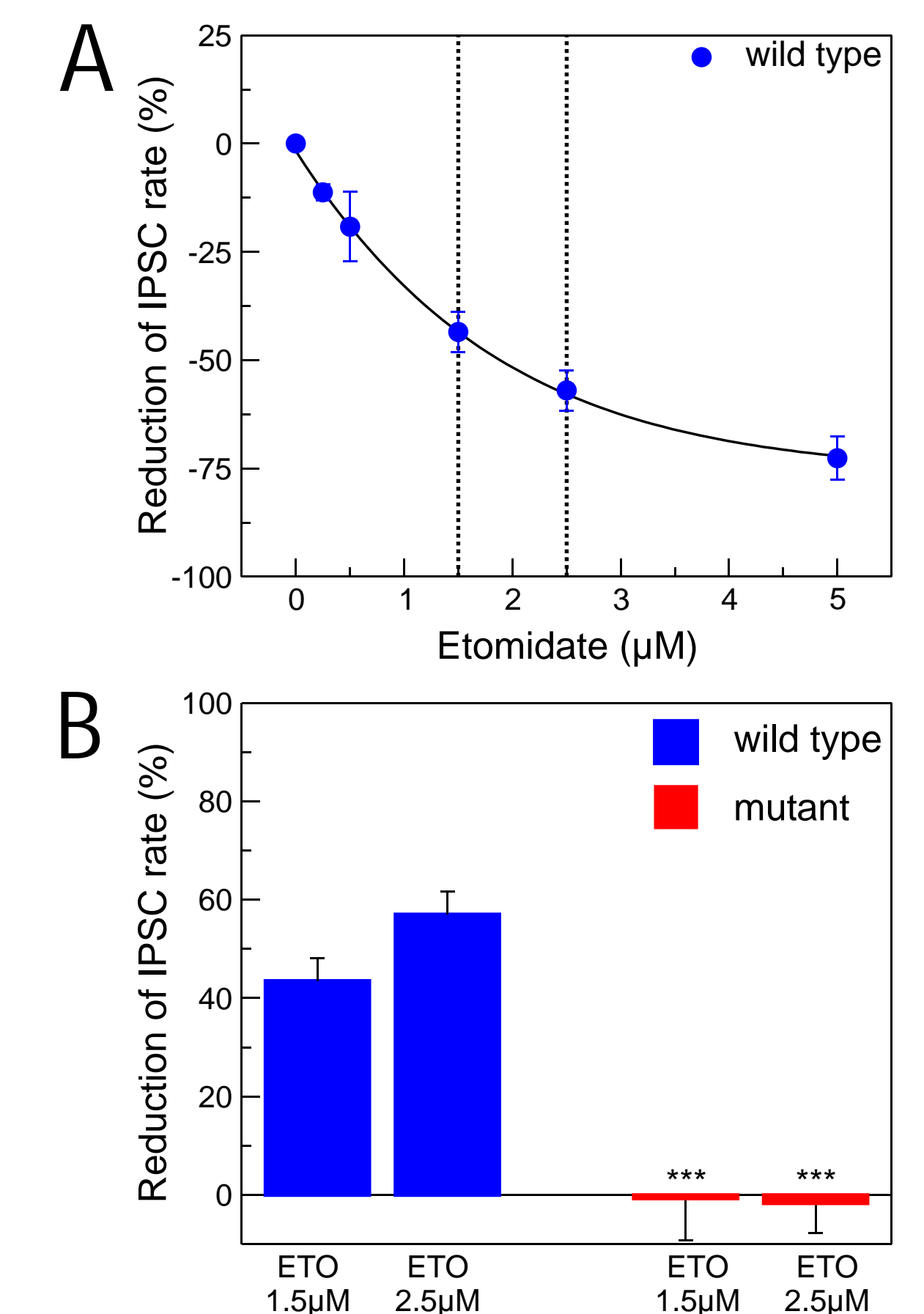
## Results



**Fig. 4:** Effects of etomidate on action potential-dependent GABA(A) inhibitory post synaptic currents (spontaneous IPSCs). CNQX 50  $\mu$ M, AP5 50  $\mu$ M, and strychnine 1  $\mu$ M were added to the bath medium. The neuron was held at a membrane potential of -70 mV. Effects of etomidate on half-decay times of spontaneous IPSCs in the **wild type** are displayed in (A). Comparison between the effects of 1.5  $\mu$ M and 2.5  $\mu$ M etomidate on half-decay times in the **wild type** and on **beta3 (N265M)** mice are demonstrated in (B). Between six and ten neurons were investigated for each data point.



**Fig. 5:** Effects of etomidate on amplitudes of spontaneous IPSCs in the **wild type** (A). (B) depicts a comparison between the effects of 1.5  $\mu$ M and 2.5  $\mu$ M etomidate on amplitudes in the **wild type** and on **beta3 (N265M)** mice. Between six and ten neurons were investigated for each data point.

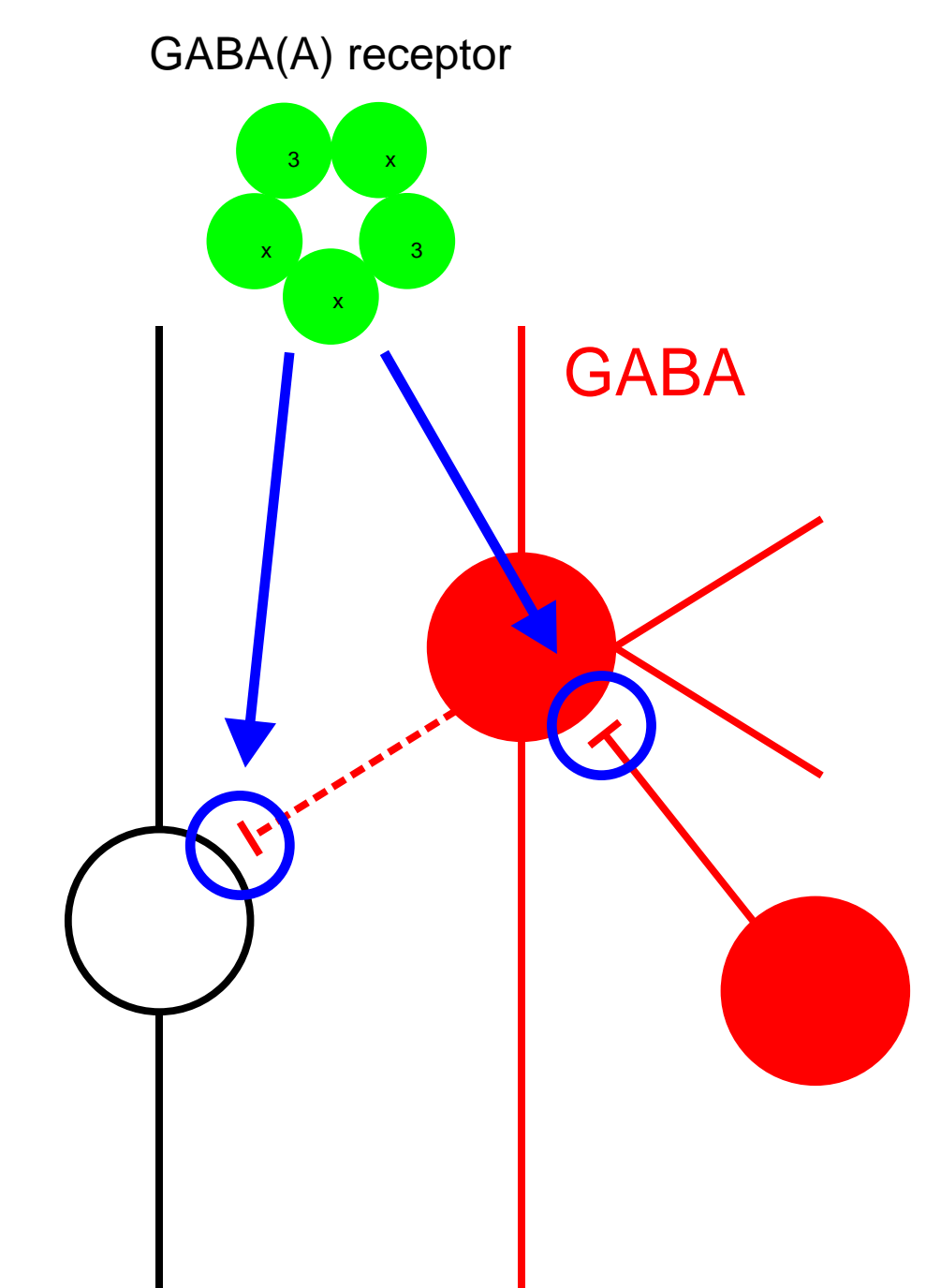


**Fig. 6:** Effects of etomidate on frequencies of action potential-dependent GABA(A) inhibitory post synaptic currents (spontaneous IPSCs). Effects of etomidate on half-decay times of spontaneous IPSCs in the **wild type** are displayed in (A). Comparison between the effects of 1.5  $\mu$ M and 2.5  $\mu$ M etomidate on half-decay times in the **wild type** and on **beta3 (N265M)** mice are demonstrated in (B).

## Conclusions

The mutation affected the frequency and the amplitude of sIPSCs as well as IPSC current decays.

We conclude that pre- and postsynaptic GABA(A) receptors incorporate beta3 subunits in spinal ventral horn interneurons.



**Fig. 7:** Schematic drawing showing a network of interneurons in the ventral horn of the spinal cord. GABAergic interneurons (red filled circles) e.g. Glutamatergic are connected to each other and decrease thereby the amount of GABA that is released at a synapse (red dotted line). Furthermore, GABAergic interneurons inhibit other, e.g. glutamatergic interneurons, as illustrated by the black circle.